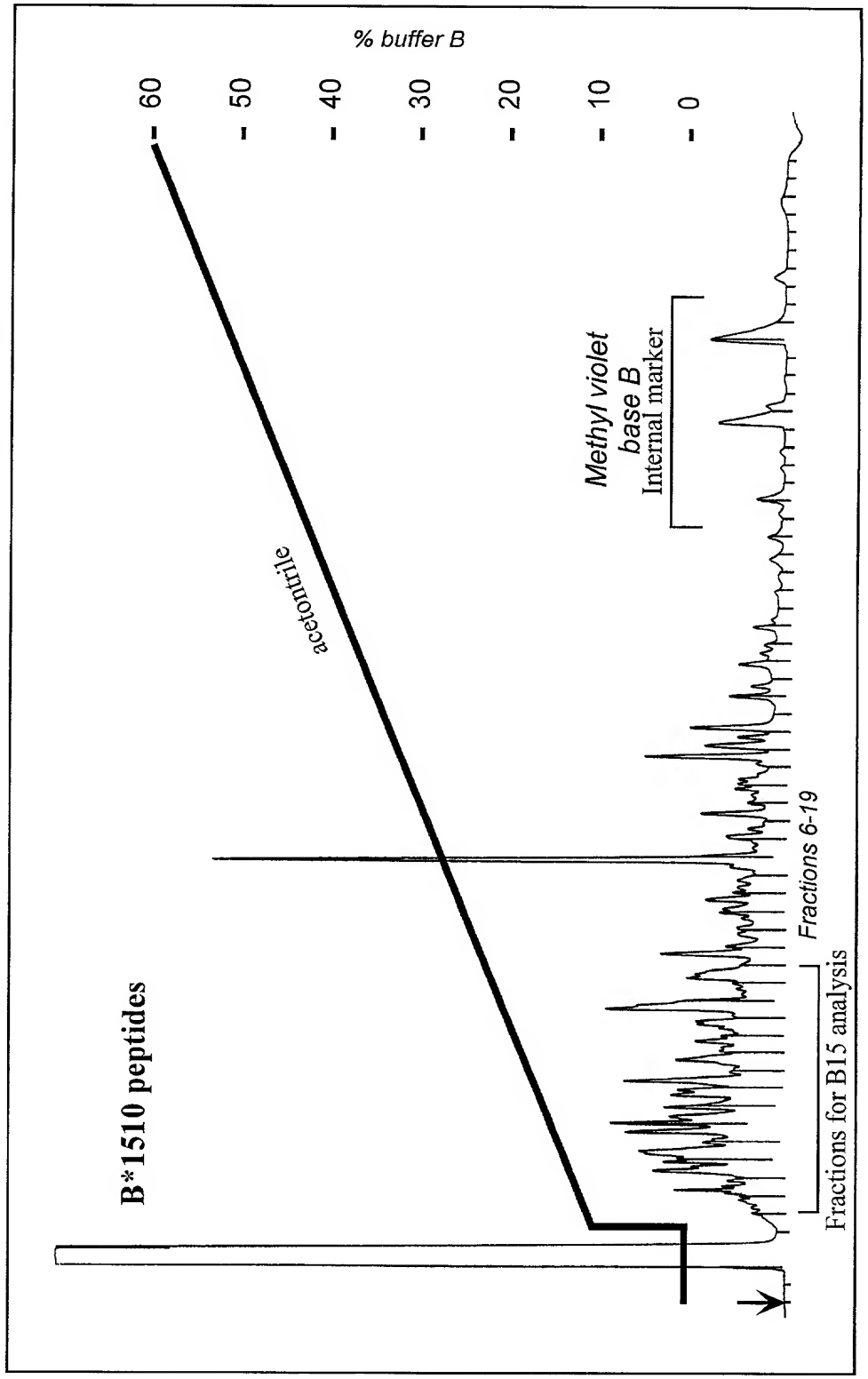

Reverse phase HPLC of class I HLA eluted peptide ligands
% buffer B
60
50
40
30
20
10
0
acetonitrile
Methyl violet
base B
Internal marker
B*1510 peptides
Fractions 6-19
Fractions for B15 analysis
UV Absorbance
Fraction Number

Fig. 1

Ion maps of peptides eluted from various B15 class I sHLA molecules. Mapping was accomplished with a nano-spray needle and an ESI mass spectrometer. The figure shows that the same ion peak is present in 3 of 4 B15 class I.

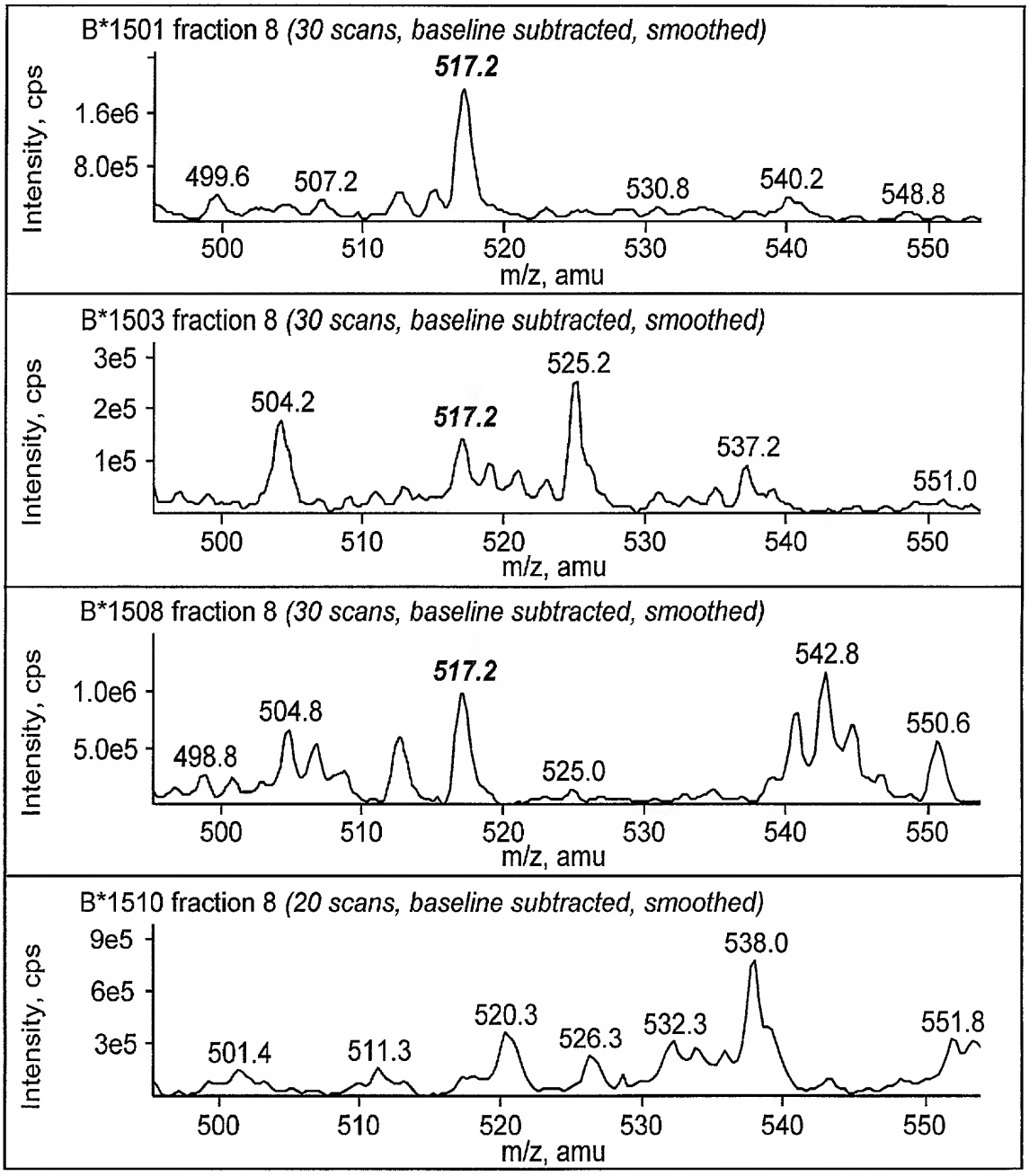


Fig. 2

MS/MS fragmentation-sequencing of ion 517.2 from the various B15 class I sHLA molecules. This data was accomplished by completing a second nanospray of the peptides in fraction 8 from the HPLC. This demonstrates how ions can be MS ion mapped and subsequently MS/MS sequenced. There is sufficient peptide present to do multiple MS/MS fragmentation runs. There is also sufficient peptide present to facilitate a submotif on fraction 8 or further separation in the event that two peptides had mapped at 517.2 in the ion map.

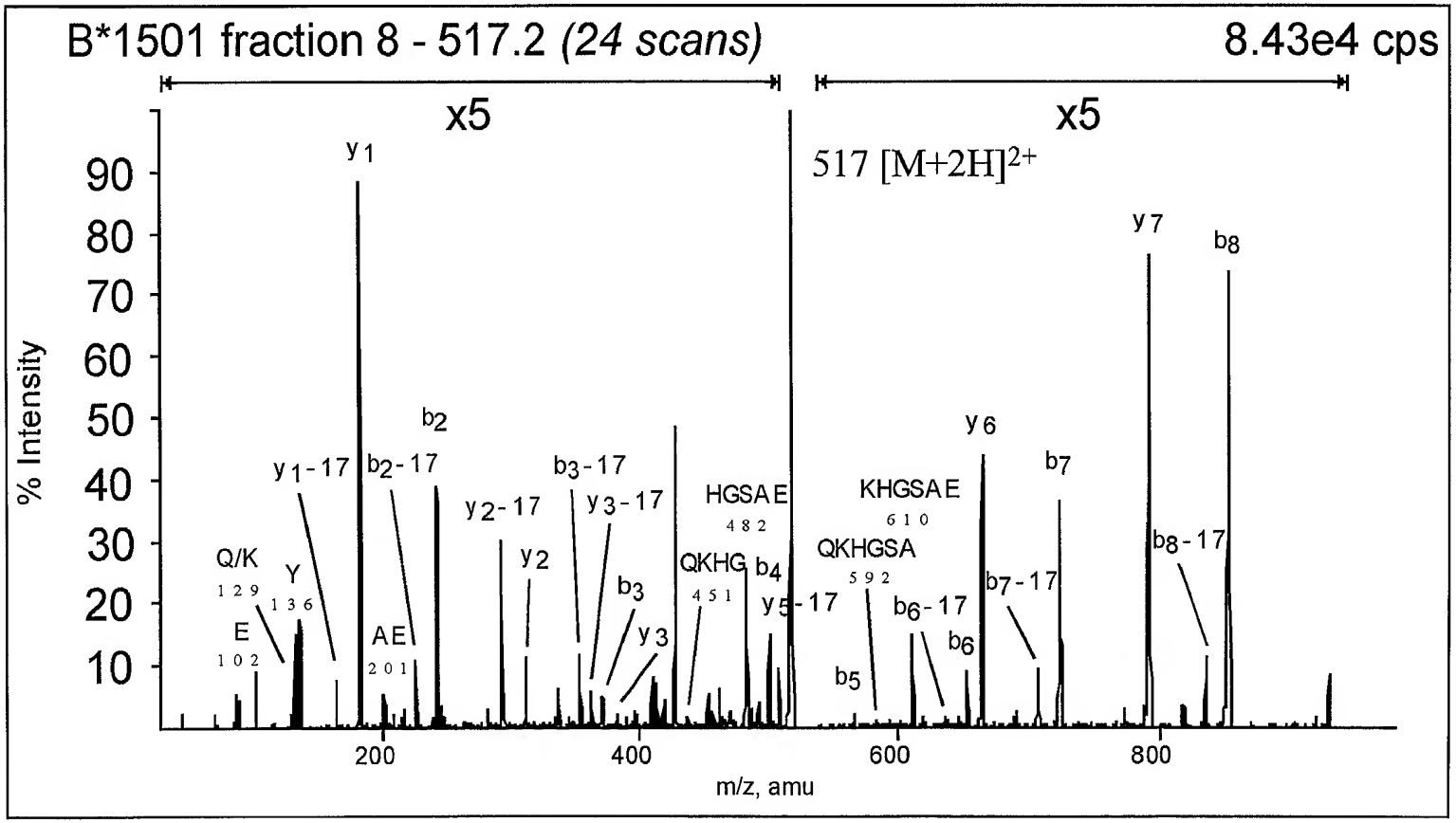


Fig. 3 1 of 3

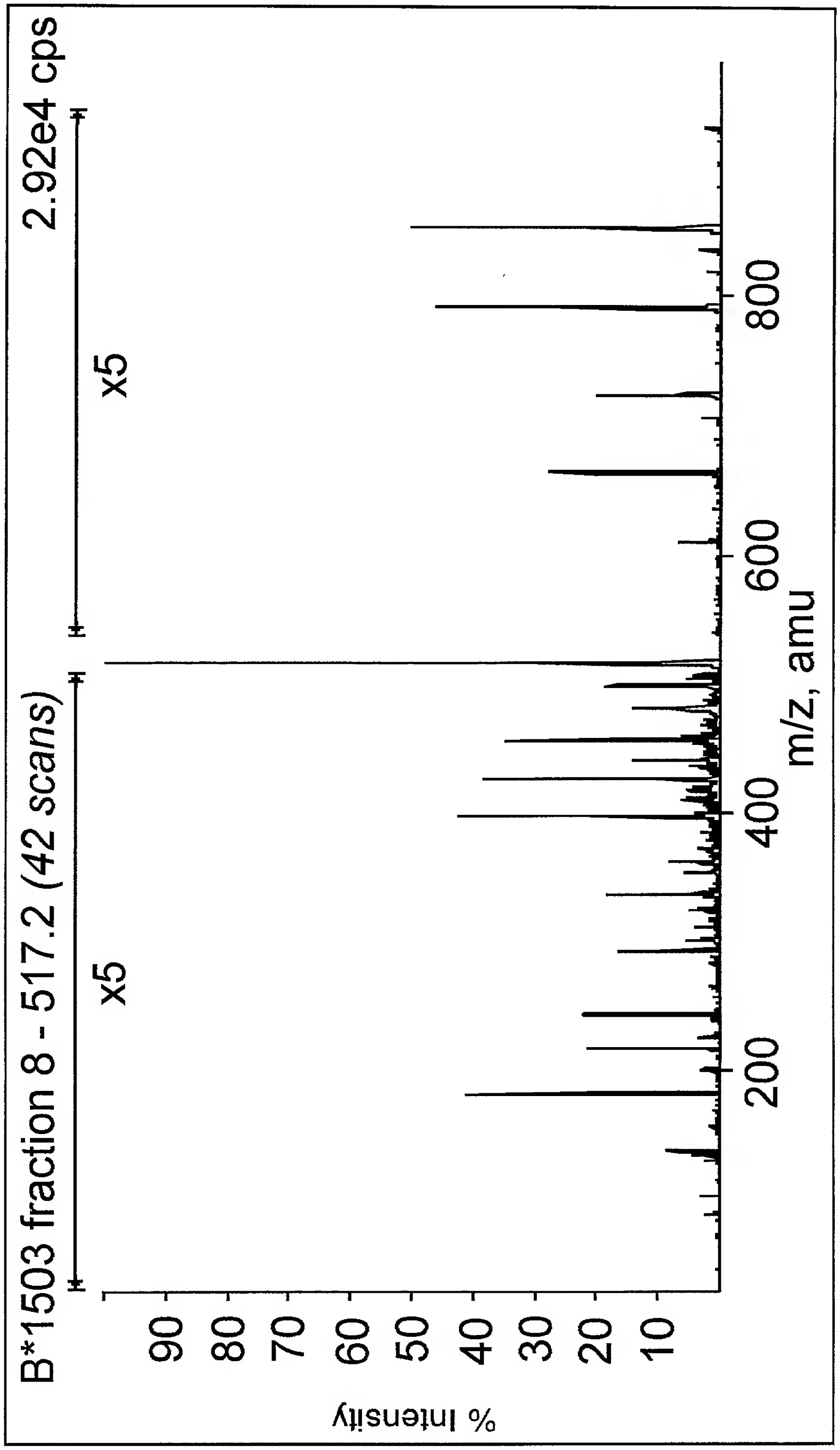
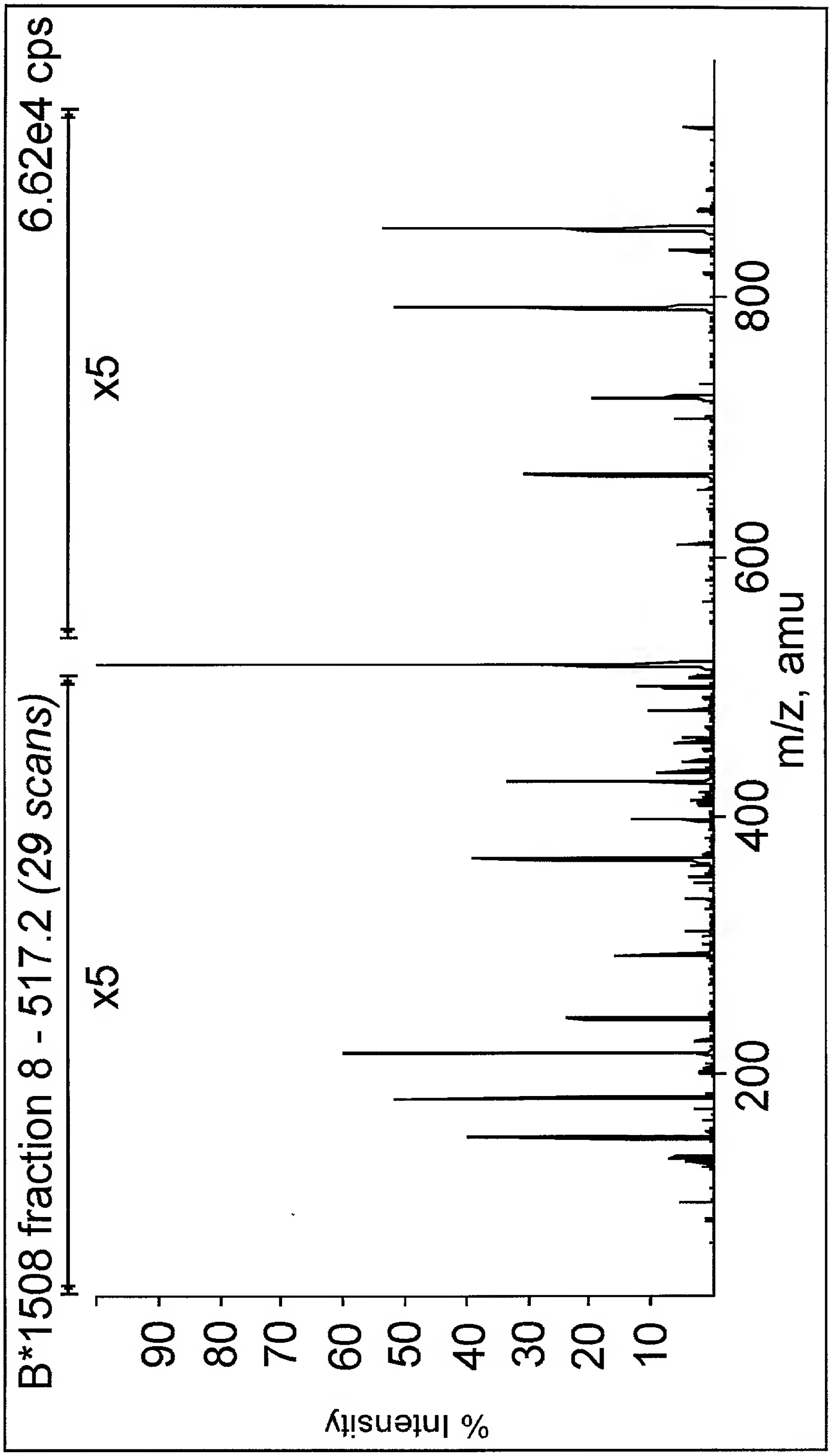


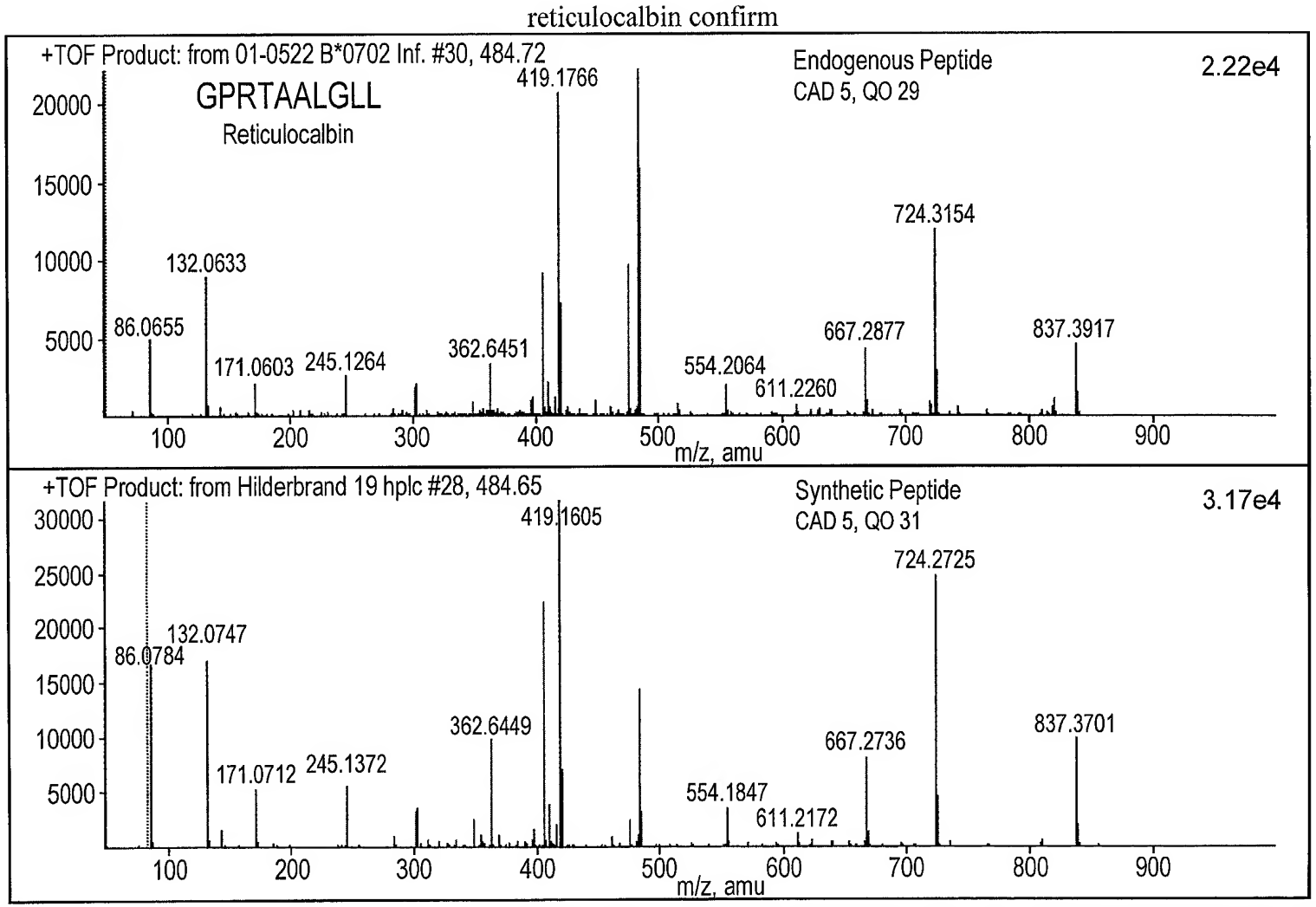
Fig. 3 2 of 3



B*1508 fraction 8 - 517.2 (29 scans)
6.62e4 cps
x5
x5
% Intensity
90
80
70
60
50
40
30
20
10
200
400
600
800
m/z, amu

Fig. 3 3 of 3

Serial No. 10/082,034 Dkt. No. 6680.040
Title: SOLUBLE HLA LIGAND DATABASE UTILIZING PREDICTIVE ALGORITHMS AND METHODS OF MAKING AND USING SAME
Applicant: William Hildebrand Group No.: 2171
Filed: 02/21/2002 Examiner: Unknown
Atty: Douglas J. Sorocco Tel: (405) 478-5344



sHLA B*0702 was secreted from HIV infected and uninfected cells. The ion maps of the peptides eluted from sHLA B*0702 in infected and uninfected cells were compared. Ion 484.72 was unique to the HIV infected cells. Ion 484.72 was subjected to MS/MS fragmentation-sequencing. We called GPRTAALGLL as the sequence of the ligand. We synthesized this peptide and found that it generated the same MS/MS fragmentation pattern as the ligand from HIV infected cells. This MS/MS data on a synthetic ligand matches our experimental data and validates the accuracy of our sequence.

Fig. 4

B*1508

	1	2	3	4	5	6	7	8	9
dominant	-	P	N	-	-	-	-	-	Y
		A	F						
			K						
			R						
			H						
			Y						
			I						
strong	-	-	-	E	H	R	-	-	F
				D					
				G					
				S					

B*1501

	1	2	3	4	5	6	7	8	9
dominant	-	Q	K	-	-	-	-	-	Y
			F						
			N						
			R						
			Y						
strong	-	M	P	P	G	-	-	-	F
		L	H	D	I				
		V	A	G					
				E					

B*1510

	1	2	3	4	5	6	7	8	9
dominant	-	H	Y	P	-	-	-	-	L
			A						
			I						
			K						
			S						
			F						
			L						
			V						
			T						
strong	-	-	G	R	R	I	M	-	F
			D	D	V		L		
			E	K					
			M	E					
			N						

Fig. 5

--WDRHTXF	AQYAAGESFY	ILGPPGSVY
-----YT	TPHTZHDEY	XLGDVNMY
XAZYEHTY	YM---FMY	AP---XVSY
NGXAMHWTY	MVGXXPAT	YMSGXYGTF
VPCGZZSY	DPHYVSGHZF	---AVVAZSY
TAZXHRGY	VVACV---Y	MPAGYNNVY
NQZHGSAEY	PLA-N-HTY	XPVVPAAZTY
TG----AY	VVAPITTGY	YMIDPSGVSY
-P--GZDEV	XAXYRRMY	
NGYDGPNAGY	PXAMQXYTY	
TPXGEPYZSY	-P-MPGXAY	
FVSNHAY	--TZSNAY	
SQFGGGSQY	MAAMVGVAVY	
SQFDHVTY	LPHQPLATY	
XAN--VT	FVTXNXEEY	
VDX---Y	GPZVMZHGY	
CPLSCFT	FQARXTEY	
FLZAMZSTY	AAAXV---VTY	
TVXDSZTHY	XPEMGZFSY	
DPSGTZSGF	YV--VR-VF	
-----PGFY	AAPVGAXESY	
-A-PHPMGY	YVA---PAF	
AQTVGYGEY	VGY--AHPGF	
------SVY	------STY	
TGNCSGTGTY	SPTYTHAVAF	
AQVNPSXTY	MPA--MVMAF	
TP--ARAPT	XA---SYTY	
SPGAETRAXY	VGYVDDTQF	
YX---RTF	ZATNSVTSTY	
YXG---GAF	YATAGEMMAF	
-P---PSSGY	TARVXSVEY	
TP---GRMY	MPAADYEVAF	
PMFDZZVY	AAFCG---XV	
AQEHGCAAZF	SPNEDXMZVF	
-M---GVHDY	VAATAGAVF	
YVS--RNZY	XLH--ET	

--WDRHTXF	FLZAMGSTY	YMVT---F
-----YT	GQYVVZPTY	APAV---VGY
ALGA--RGY	PMFDPPZTF	------TGF
XS---VEY	XAVGHSGGTY	PVPNVRXNY
AQFASGAGZ	-----PTY	------TXSX
-G--CDY	-----PSY	YMVCNAEEY
---ZARGY	EPAMVXZCF	-----XRDXY
ALNGRVTMY	XAHTECPRGY	SLX-----F
DPHAPPZY	VQGPVGVZY	ALGSZAXMPF
XAZVZMTAY	TGAPVSEEGY	VGYVDDTQF
NQZHGSAEY	VQXYYGSVV	DVEGWMSZY
FGXACXATSY	GQPGAPXGGZY	AQHPSAXRF
APMARGZY	GPPHNGXRAY	-----ZAY
TG----AY	AAHWHVEAY	GLGZTSAEF
GQZZAVDF	TPPTRRESY	NAXG--RESSF
TPXGEPYZSY	FPTDRRSZF	TARVXSVEY
GQHASVXSY	YTGVSYXHF	AAFCG---XV
FVSNHAY	AQASAPDAY	XLH--ET
---------SY	GQRKGAGSVF	ILGPPGSVY
NPPAZZPN	VQYYXPF	XLGDVNMY
-Q-DPPPDMZY	ATGTAZNXNZY	VMGXTNANF
XQ--AGGZY	VVACV---Y	AVVTXZSDF
SQFGGGSQY	PLA-N-HTY	AMNPTNTVF
SQFDHVTY	VVAPITTGY	SQXAAGVDVF
--AAHVPPGY	XQYTVGYF	-VFVSHTTF
FMDVGAPTVY	PLFGZTAGZY	XQGHHEMFY
XAN--VT	A----ZXEY	PQGZMA--Y
AQM--SEY	VAGGW---F	HLTGNEATSF
CPLSCFT	SGAXDRAYZF	YMIDPSGVSY
FLZAMZSTY	VQGPVGTDF	
TVXDSZTHY	FQARXTEY	
AQAAPFAGY	XAGFFXXEY	
----FGHY	XQ----ZY	
ALW--PZF	FQ----TX	
VPHZNAY	TP---AZAF	
-----GHGGY	VVATZNZZX	

SVSXPHAP
APFTGGNGX
EHVASSPAL
HHAPCGVSX
NHAIVSTSV
GHZNSVTSV
SHZAPCTSV
FVARFVSX
HHSDGSVSL
MCZ-GMPAX
GHGANNDPAX
XHSZPAGPAX
XHVVS--VX
YHGSZNPEX
--------M
EHGXENGH
AHZAPPPTX
FTACZNPAX
SHAGAGXVX
GHXEGPXX
XHGGDHVX
YHHDXVX
MAGAWCRX
FH--XXX
EH---TVX
MAX----VV
------PVX
XHYDRNZX
------AXSV
XHWPVNEX
-H-----PVF
XHEVZPXHX
-HGCPGMPX
ETPEHAPVX
MXPGNSAXYX

Fig. 5 continued

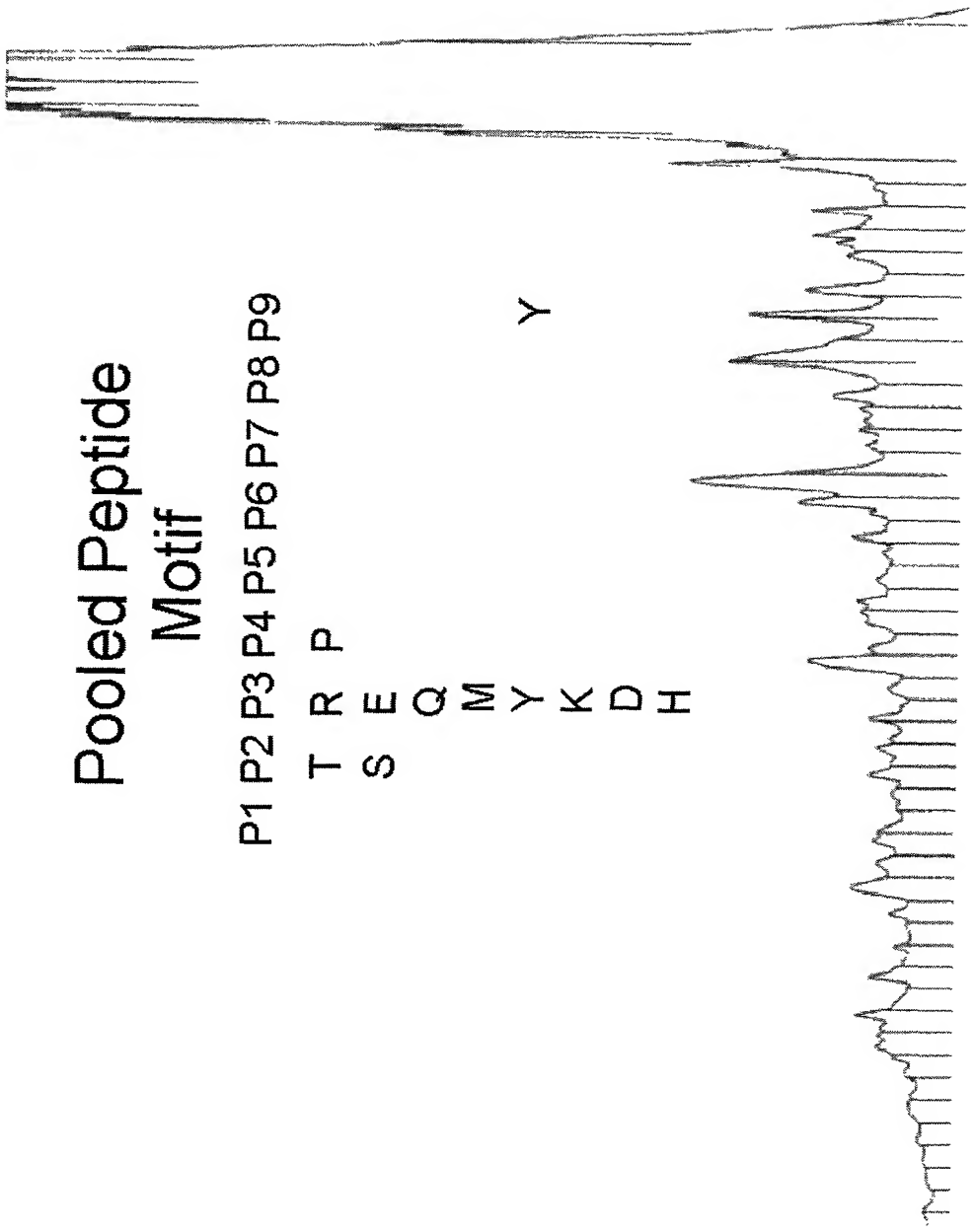


Fig. 6

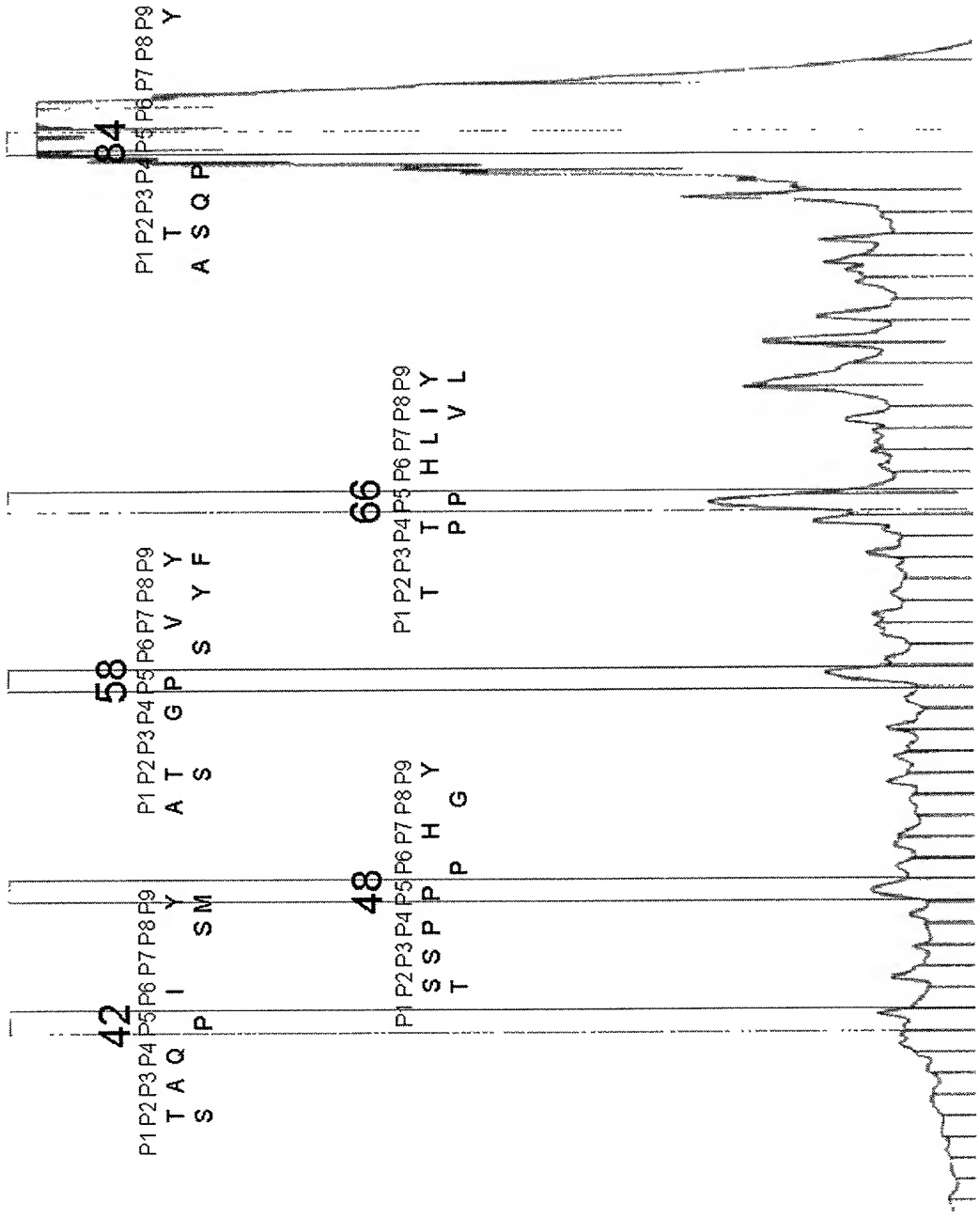

P1 P2 P3 P4 P5 P6 P7 P8 P9
84
T A S Q P Y
66
T T P P H L I Y V L
58
A T G P S V Y Y F
48
S S P P T H P G Y
42
T A Q S P I S M Y

Fig. 7

Narrowing search parameters using fraction motifs:

Ovarian Carcinoma Immunoreactive Antigen

MNGRADFREP	NAEVPRPIPH	IGPDYI**PTEE**	**ERRY**FAECND	**ESFWFRSY**PL
AATSMLITQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSYVKTCQE
KFKKLENSPL	GEALRSGQAR	**RSSPPGHYY**Q	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SA**PTGITDHI**	**Y**QGPDPNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNKYG
DTWDE				

Scanning with whole-pooled motif revealed 4 putative epitopes.

Ovarian Carcinoma Immunoreactive Antigen

MNGRADFREP	NAEVPRPIPH	IGPDYIPTEE	ERRYFAECND	ESFWFRSYPL
AATSMLITQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSYVKTCQE
KFKKLENSPL	GEALRSGQAR	**RSSPPGHYY**Q	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDPNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNKYG
DTWDE				

Scanning with fraction 48 peptide motif revealed 1 putative epitope.

Fig. 8

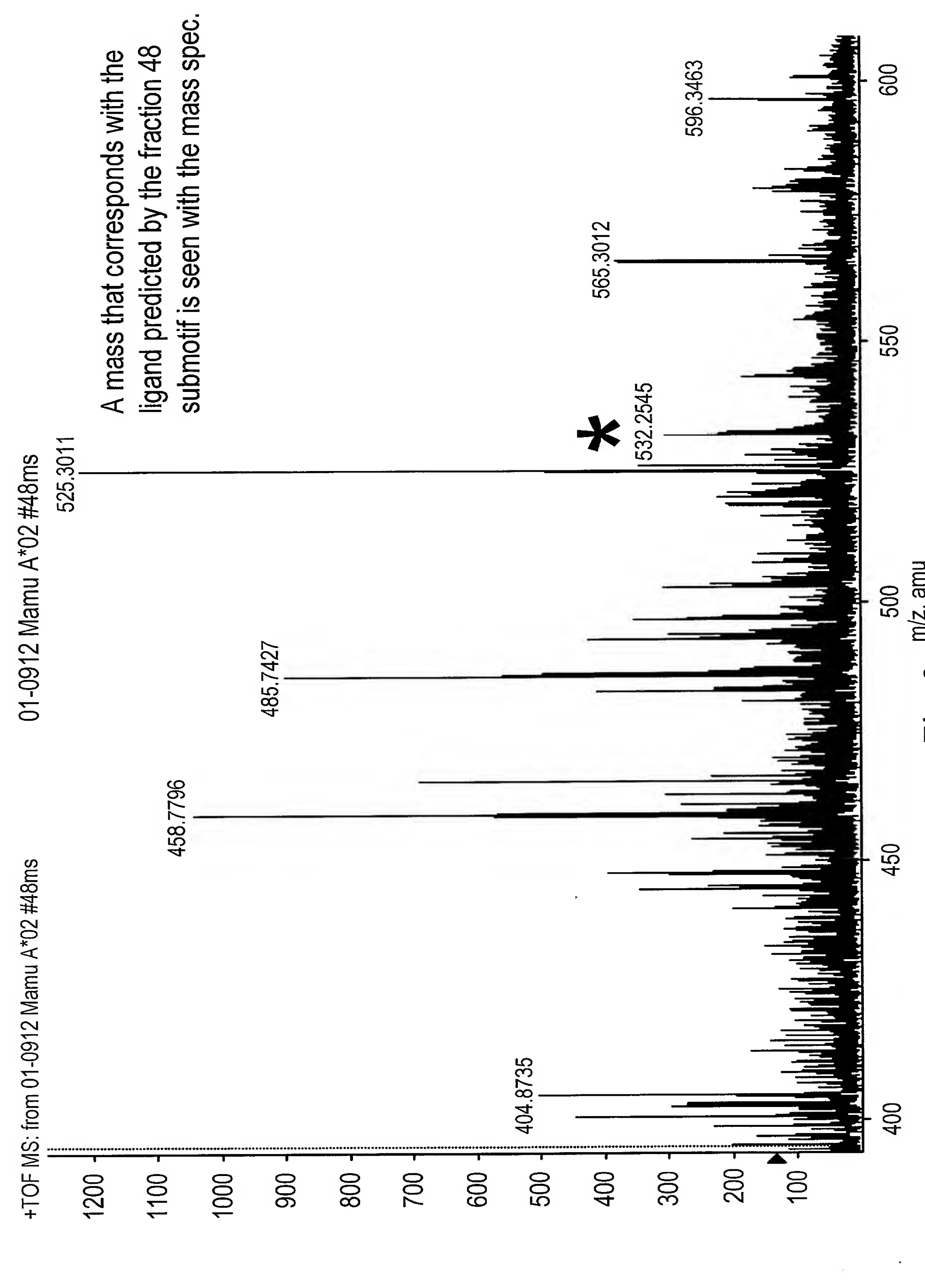

+TOF MS: from 01-0912 Mamu A*02 #48ms
01-0912 Mamu A*02 #48ms
A mass that corresponds with the ligand predicted by the fraction 48 submotif is seen with the mass spec.
404.8735
458.7796
485.7427
525.3011
532.2545
565.3012
596.3463
*
100
200
300
400
500
600
700
800
900
1000
1100
1200
400
450
500
550
600
m/z, amu

Fig. 9

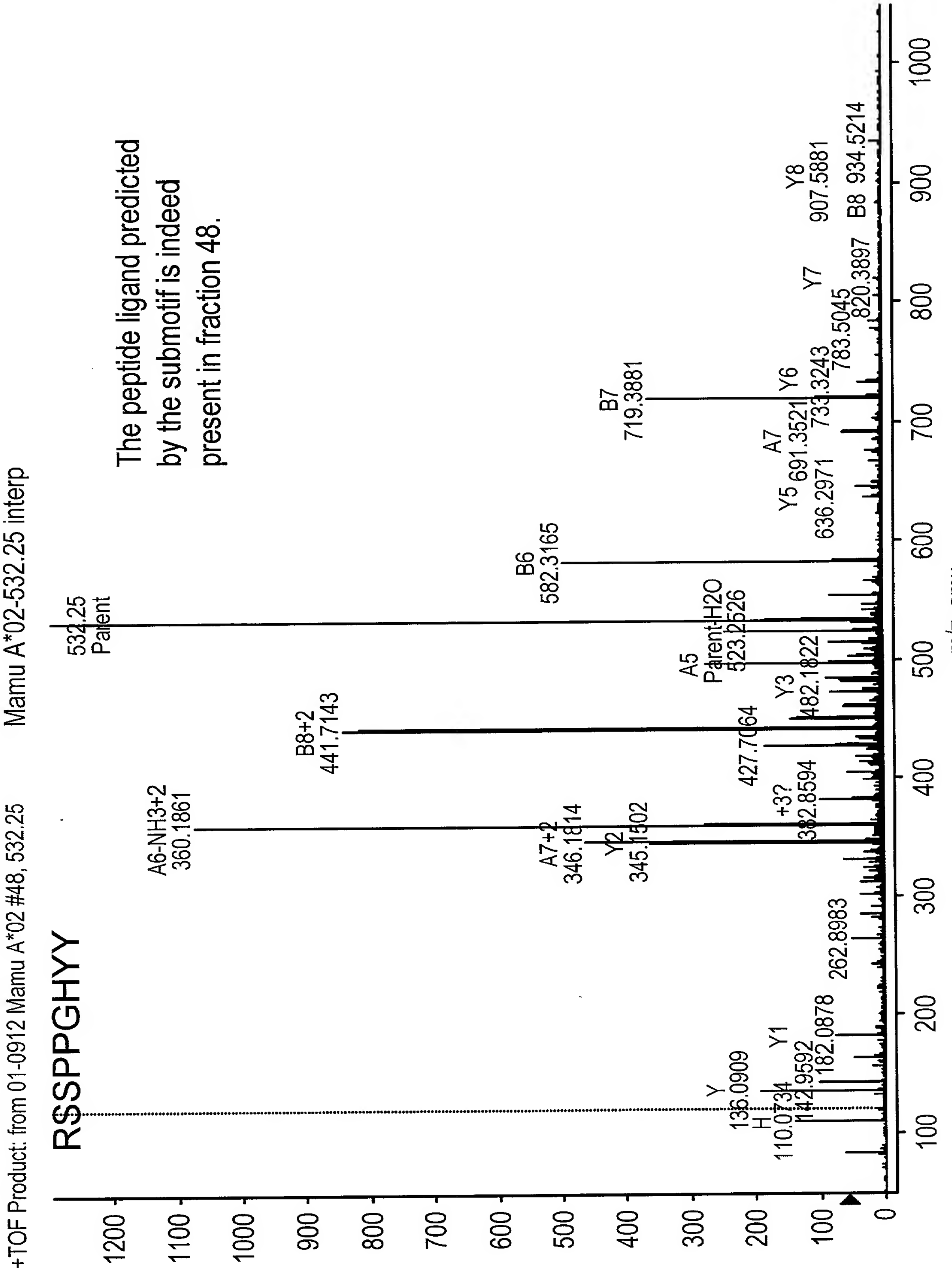

+TOF Product: from 01-0912 Mamu A*02 #48, 532.25
Mamu A*02-532.25 interp
RSSPPGHYY
The peptide ligand predicted
by the submotif is indeed
present in fraction 48.
A6-NH3+2
360.1861
B8+2
441.7143
532.25
Parent
B6
582.3165
B7
719.3881
A7+2
346.1814
Y2
345.1502
427.7064
Y3
482.1822
A5
Parent-H2O
523.2526
Y5
636.2971
A7
691.3521
Y6
733.3243
783.5045
Y7
820.3897
Y8
907.5881
B8 934.5214
Y
136.0909
H
110.0734
142.9592
Y1
182.0878
262.8983
+3?
382.8594
m/z, amu

Fig. 10

Motif Data (Edman sequencing)

	1	%	fold	2	%	fold	3	%	fold	4	%	fold	5	%	fold	6	%	fold	7	%	fold	8	%	fold	9	%
Dominant 3.5 fold increases or more over prior round							F	9.20	11.18																	
							I	7.60	7.01																	
							N	6.20	4.11																	
							M	4.90	10.42																	
Strong 2.5-3.5 fold increase over prior round	K	31.50		R	53.80	2.57	Q	8.20	3.10	P	8.30	2.97													L	7.50
	R	15.50					K	5.40	2.67																	
	S	10.40					L	5.10	3.47																	
Weak 2.0-2.5 fold increase over prior round							A	5.80	2.08				M	4.90	2.28				T	7.70	2.12					
Trace 1.50-2.0 fold increase over prior round				Q	3.60	1.79	P	2.20	1.94	K	11.00	1.63	I	6.50	1.99							Q	11.40	1.93		
										S	6.00	1.68	F	4.20	1.83							N	5.70	1.55		
										V	5.30	1.99	H	1.80	1.67											

Fig. 11

Design of HLA Ligand/Motif Database

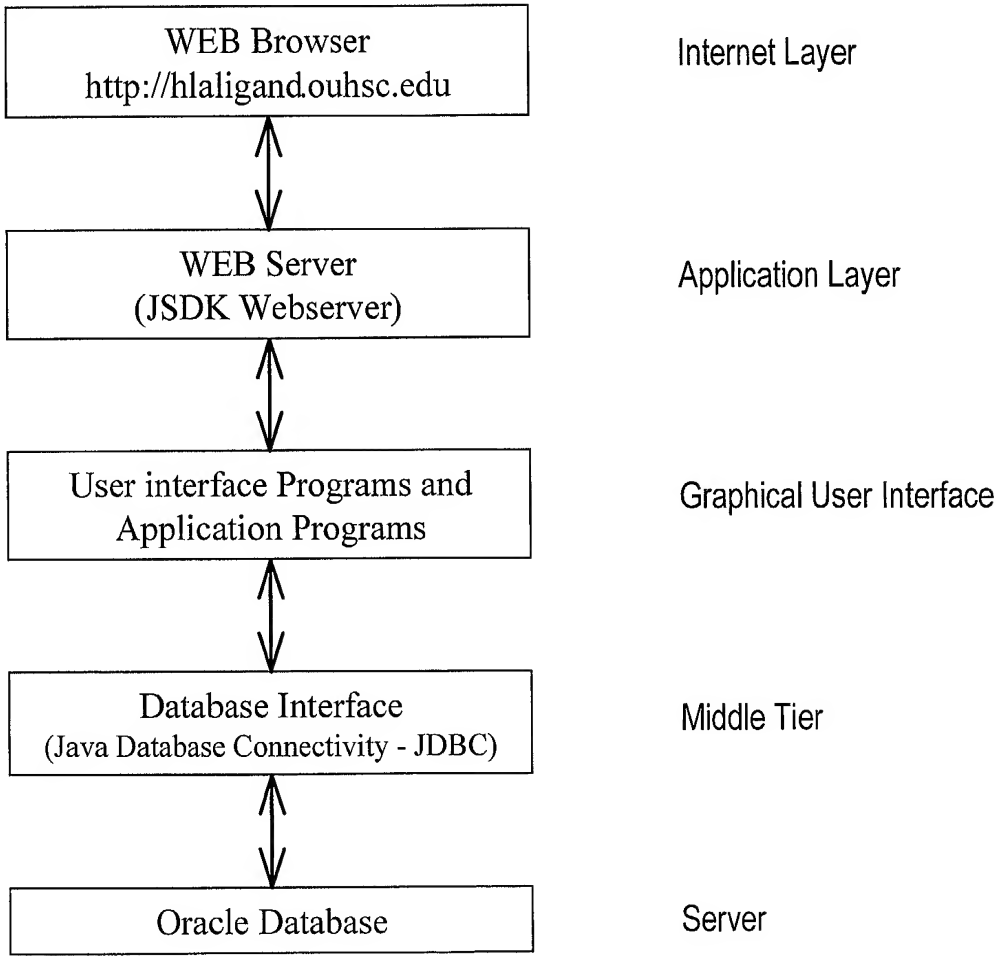


Fig. 12

Entity-Relationship (ER) Diagram for HLA Ligand/Motif Database

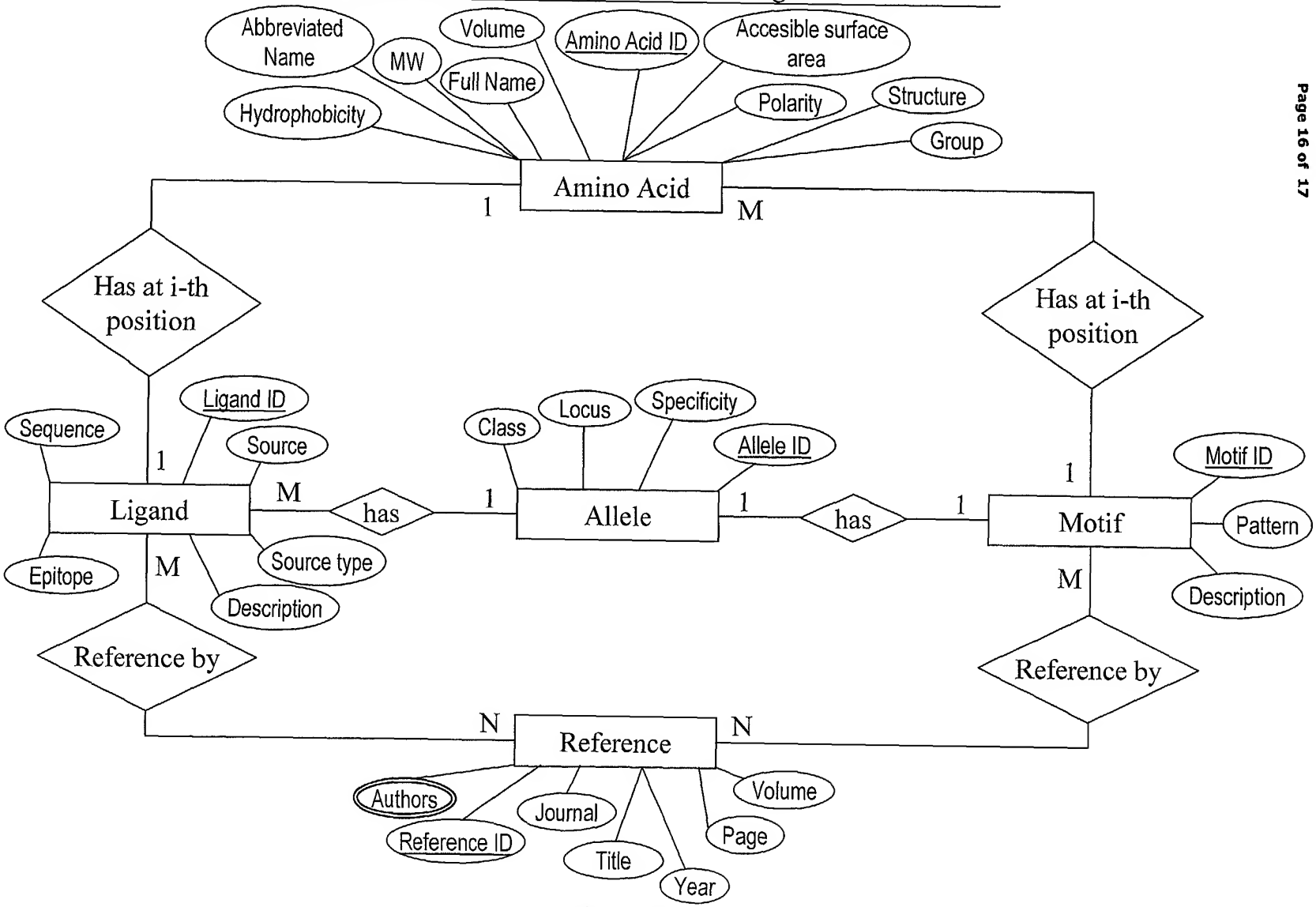


Fig. 13

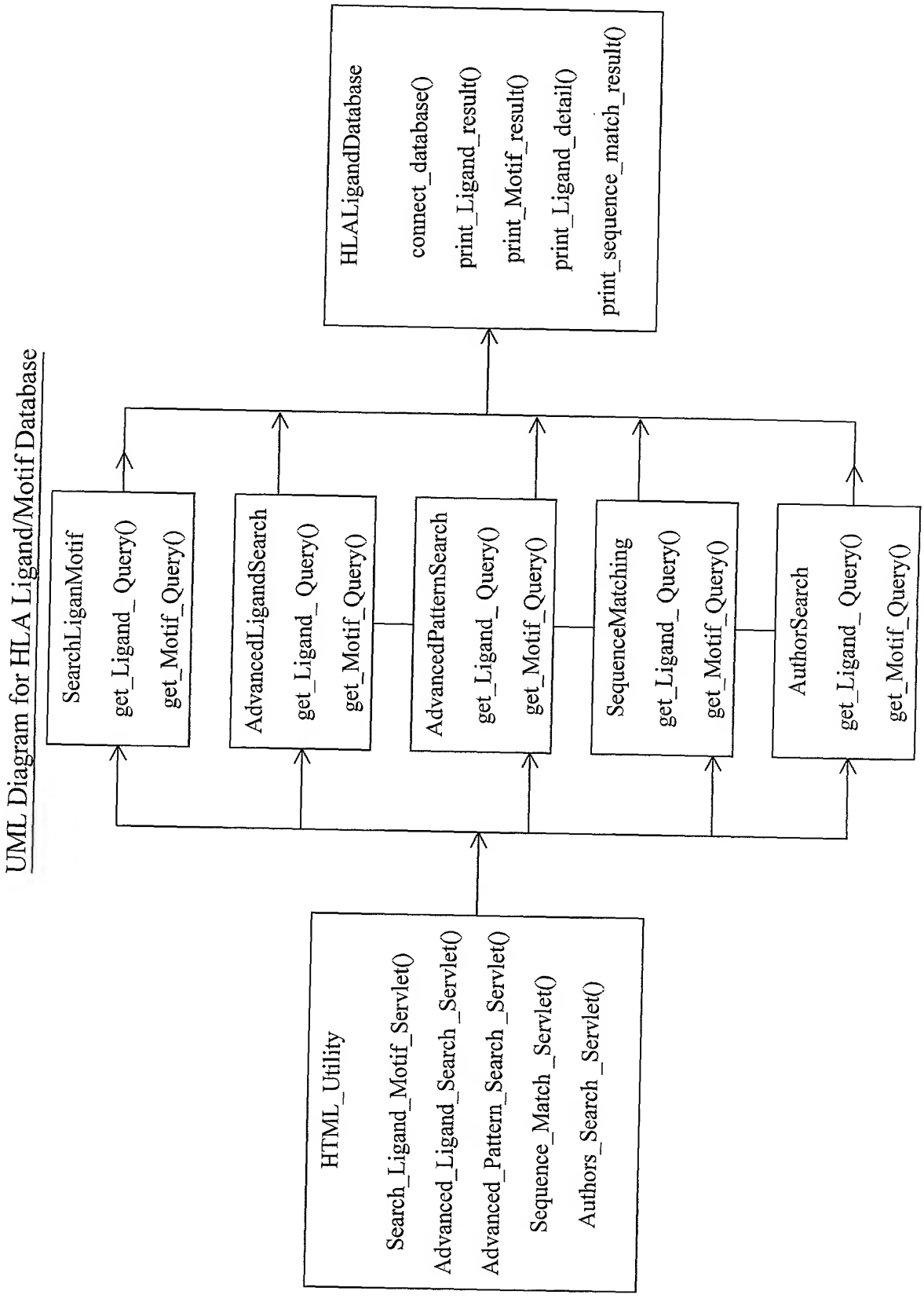


Fig. 14